UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/694,520	10/27/2003	Gail Bishop	17023.019US1	2261

53137 7590 04/10/2007
VIKSNINS HARRIS & PADYS PLLP
P.O. BOX 111098
ST. PAUL, MN 55111-1098

EXAMINER	
MCGILLEM, LAURA L	
ART UNIT	PAPER NUMBER
1636	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	04/10/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/694,520	BISHOP ET AL.	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 January 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2-4,6,7,11,12,14-20,22-25,28 and 31-52* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2-4,6,7,11,12,14-18,20,22-25,28,31-41 and 43-52* is/are rejected.

7)☒ Claim(s) *19 and 42* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *10/27/2003* is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

It is noted that claims 1, 5, 8-10, 13, 21, 26-27 and 29-30 are cancelled, claims 2-4, 6-7, 11-12, 14-16, 18, 20, 28, 31-32 have been amended and claims 33-52 have been added in the amendment filed 1/16/2007. Claims 2-4, 6-7, 11-12, 14-20, 22-25, 28, 31-52 are under examination.

In the Office Action mailed 10/2/2006, claims 18, 19, 26 and 27 were previously indicated as allowable if re-written in an independent form. As suggested, claims 18 and 19 have been amended. However, on further consideration, new grounds of rejection have been applied (see below).

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 10 has been canceled and claim 32 has been amended to remove the phrases "weak promoter" and "modified Rous sarcoma virus (RSV) promoter". Therefore, the rejection of claims 10 and 32 under 35 U.S.C. 112, second paragraph has been withdrawn.

Claims 43-50 (new) are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 43 is vague and indefinite because it recites the phrase "weak promoter" and the metes and bounds of the weakness of the promoter required are not clear so

that the skilled artisan would know whether a particular promoter would meet the limitation of the claims.

In response to a similar rejection for now amended claim 32, Applicants submit that the specification at page 17, lines 8-15 defines a weak promoter as "i.e., a promoter that controls expression of the toxin in such a manner as to prevent the toxin from killing cells before the gene targeting construct has had a chance to incorporate into the chromosome of the host somatic cell via homologous recombination. Examples of weak promoters are known to the art, and include, for example, a modified Rous sarcoma virus (RSV) promoter and the SV40 promoter." Applicants submit that the metes and bounds of this term would be clear to a skilled artisan.

Applicant's arguments filed 1/16/2007 have been fully considered but they are not persuasive. While the specification does contain the above disclosure for the phrase "weak promoter", it is based on expression and activity of a toxin. Without further disclosure of the toxin, which may be a very strong or alternately a very weak toxin, it is difficult to determine whether a promoter is weak or not weak. This disclosure and disclosed examples are not a limiting definition of the promoters that can constitute a weak promoter. The word weak is a relative term that limits the promoter. "When a term of degree is presented in a claim, first a determination is to be made as to whether the specification provides some standard for measuring that degree" See MPEP 2173.05(b). Without some disclosed or claimed standard promoter strength for measuring the degree of promoter strength intended by the Applicants, the skilled

artisan would not know metes and bounds of what promoters would constitute weak promoters and meet the limitations of the claimed vector.

Claim 45 recites the limitation "the recombinase" but it is vague and indefinite because the claim is dependent on claim 43, and there is no recombinase recited in claim 43. There is insufficient antecedent basis for this limitation in the claim. It is not clear to what recombinase the claim refers.

Claim 46 recites the limitation "the first and second site-specific recombination sequences" but it is vague and indefinite because the claim is dependent on claim 43, and there are no site-specific recombination sequences recited in claim 43. There is insufficient antecedent basis for this limitation in the claim. It is not clear to what site-specific recombination sequences the claim refers.

Claims 44 and 47-50 are indefinite insofar as they are dependent on an indefinite claim.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 43-48 and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi et al (U.S. Patent No. 5,631,153) in view of Sedivy et al. (of record). This is a rejection of newly added claims.

Applicants' previous arguments to the rejection of Capecchi et al in view of Sedivy et al have been addressed in pages 7-12 of the previous Office action, mailed 10/2/2006. Claim 43 is very similar to claim 1 (now cancelled) drawn to a somatic cell gene targeting vector with the addition of the limitation of the promoter being a weak promoter or a PGK promoter. Since there is no specific limiting definition of "weak promoter" in the instant disclosure, absent evidence to the contrary, any promoters listed on Table IIB, column 14 of Capecchi meet the limitation of a weak promoter.

Capecchi et al teach a vector to modify a target DNA sequence in the genome of a cell capable of homologous recombination comprising a first homologous vector DNA sequence capable of homologous recombination with a first region of a target DNA sequence, a promoterless positive selection marker sequence, a second homologous vector DNA sequence capable of homologous recombination with a second region of the target DNA sequence, and a negative selection marker sequence (See abstract, column 5, lines 5-35, column 6, lines 42, Fig 9 description, column 9, lines 10-15, and column 26, example 6, in particular).

Capecchi et al teach that that said vector can be used in a method to inactivate genes by homologous integration of the positive selection sequence into the targeted gene and disrupt the expression of the gene in a cell, which can then be selected by way of the expression of the positive selection marker (see column 12, lines 34-52,

column 13, lines 6-25 and Example 6, for example). "The positive selection markers... may be... constructed so that homologous recombination will place it under control of the regulatory sequences in the target sequence" (column 8, lines 26-34). Absent evidence to the contrary, the vector taught by Capecchi et al could be used to target genes in somatic cells and would therefore meet the limitation of the intended use of the vector to target genes in somatic cells.

Capecchi et al do not teach that polyadenylation sequences flank the positive selection sequence.

Sedivy et al teach targeted homologous gene recombination in somatic cells using positive negative selection (PNS) vectors and using promoterless vectors (see page 88, left column, paragraph 2, bridging to right column, and Figure 1 for example). Sedivy et al teach the use of a polyadenylation sequence with the PNS and promoterless vectors and also teach that the Cre-Lox system of recombination can be used for specific recombination (see Figure 1 and 2, and page 90, left column, paragraph 2). While Sedivy et al does not specifically teach the combination of a promoterless positive selection marker in combination with a cre/lox system, Sedivy et al does contemplate use of the cre/lox system for homologous recombination to introduce a positive selection marker into a genome. Sedivy et al does teach targeted homologous gene recombination in somatic cells using positive negative selection (PNS) vectors and using promoterless vectors (see page 88, left column, paragraph 2, bridging to right column, and Figure 1, in particular). One of ordinary skill in the art

would know that the term Lox refers to the recombination recognition sequence and the term Cre refers to Cre recombinase (**claim 45**).

It would have been obvious to modify the teaching of Capecchi et al to include polyadenylation sequences and cre/lox recombinase recognition sites in the PNS vector because Capecchi et al teach that different regulatory sequences to modify gene expression can be used and combined (column 13, lines 65-67, in particular) and Sedivy et al teaches that polyadenylation sequences flank the positive and negative selection sequences in PNS vectors. Polyadenylation signals and polyA tails are well known in the art as important structures for transcription and translation. Capecchi et al do teach that the positive selection marker can in some cases comprise a polyadenylation sequence (see column 13, lines 26-49, in particular) so Capecchi et al contemplate use of polyadenylation sequences. Since Capecchi et al teach a promoterless positive selection marker in a PNS vector and methods to target genes with the vector, it would be obvious to include a polyadenylation signal at the end of each marker gene as taught by Sedivy et al, in order to get proper transcription and translation of the markers. It would also be obvious to use an SV40 polyadenylation sequence (**claim 48**) because Capecchi et al teach that SV-40 early can be used as a regulatory sequence. The motivation to do so is the benefit of being able to produce functional selection markers in order to perform the intended gene disrupting method. There is a reasonable expectation of success in creating a PNS vector with a Cre-Lox recombination site for use in gene targeting of somatic cells since this has worked previously in cited references. Therefore, the combination of the teaching of Capecchi et

al and Sedivy et al renders obvious the somatic gene targeting vector comprising a weak promoter (**claims 43-46**).

Capecchi et al teach that the positive selection markers sequences can be neomycin (Neo) and that the negative selection marker can be diphtheria toxin (see column 7, lines 18-20, Table I, in particular), which meets the limitation of **claims 47 and 50.**

Claims 2-4, 6-7, 12, 14-18, 20, 22-25, 28, 31-37, 39-41, 43-48 and 50-51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi et al (U.S. Patent No. 5,631,153), in view of Sedivy et al (of record) and further in view of Barsoum (U.S. Patent No. 4,956,288). This is a NEW rejection.

Applicants claim a method for disrupting a gene of interest into a somatic cell *in vitro* further comprising introducing a double-stranded oligonucleotide into the somatic cell. Applicants claim a somatic cell gene targeting transfection mixture comprising a gene targeting vector and a double-stranded oligonucleotide.

The teaching of Capecchi et al and Sedivy et al are discussed in the above rejection. Specifically, Capecchi et al teach that that the targeting vector can be used in a method to inactivate genes by homologous integration of the positive selection sequence into the targeted gene and disrupt the expression of the gene in a cell, which can be selected by way of the expression of the positive selection marker (see column 8, lines 26-34, column 12, lines 34-52, column 13, lines 6-25 and Example 6, for example). Capecchi et al disclose the targeting methods for endothelial cells from patients (see column 16, lines 41-65, for example), which reads on somatic cells.

Capecchi et al also disclose an embodiment of a method comprising electroporation to introduce the vector into cells (see column 24, lines 11-16 and column 26, lines 27-32, for example). Sedivy et al teach targeted homologous gene recombination in somatic cells using positive negative selection (PNS) vectors and using promoterless vectors (see page 88, left column, paragraph 2, bridging to right column, and Figure 1, in particular).

Capecchi et al and Sedivy et al do not teach a method comprising the step of introducing a double-stranded oligonucleotide into the somatic cell along with the targeting vector.

Barsoum teaches methods of integrating foreign DNA into mammalian cell lines at a high copy number using electroporation (see column 3, lines 55-60). Barsoum teaches that carrier DNA is added to the transfection mixture for electroporation in order to increase the transfection efficiency. Barsoum teaches a preferred embodiment in which the carrier DNA is ~300 bp to 1000 bp in length and that sonication may be used to prepare DNA with the desired size distribution (see column 9, lines 44-65, for example). Absent evidence to the contrary, the DNA taught by Barsoum would be double stranded, since Barsoum does not disclose a step to render the DNA single stranded. Although the instant specification discloses embodiments in which the limitation of oligonucleotide is about 200 bp, or typically less than 200 bp, at least 100 bp, at least 75 bp, at least 50 bp or at least 20 bp, the specification defines oligonucleotide as "a short polynucleotide or a portion of a polynucleotide". Therefore the carrier DNA at approximately 300 bp in length meets the limitation of a double

stranded oligonucleotide. Barsoum teach that foreign DNA, carrier DNA and Hepes buffer combined in a mixture with cells and transferred to an electroporation container (see column 10, lines 65-67 bridging to column 11, lines 1-10, in particular), which meets the limitation of a transfection mixture.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teaching of Capecchi et al and Sedivy et al and make a transfection mixture comprising a targeting vector and a short carrier DNA oligonucleotide in order to perform a method to disrupt a gene of interest in a somatic cell comprising the step of introducing a short carrier DNA oligonucleotide with the targeting vector in a somatic cell because Barsoum teaches that the efficiency of transfection will be increased by adding a high DNA concentration. The motivation to add a double stranded oligonucleotide is the expected benefit of increasing the total number of integration events and the frequency of high copy number integration events as suggested by Barsoum (see column 10, lines 44-50, in particular). There is a reasonable expectation of success of a method comprising the step of adding a double stranded oligonucleotide in a method to introduce a targeting vector into a cell because it has worked in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, the combination of the teaching of Capecchi et al, Sedivy et al and Barsoum renders obvious the method for disrupting a gene of interest into a somatic cell *in vitro* further comprising introducing a

double-stranded oligonucleotide into an *in vitro* somatic cell (**claim 18**). The combination of the teaching of Capecchi et al, Sedivy et al and Barsoum further renders obvious the transfection mixture of **claim 32**.

It would have been further obvious to introduce a Cre recombinase to the cell after the promoterless PNS vector has been introduced in order to be able to use the loxP sites to remove the positive selection marker because Cre recombinase is functional at the lox P site. Sedivy et al teaches the advantage of using the CreLox system of site-specific recombination especially when sequential targeting might be desired. The motivation to use a site-specific recombination system like Cre/lox with a recombinase is the expected benefit of being able to remove the positively selectable marker from the genome if desired. Therefore, the combination of Capecchi et al, Sedivy et al and Barsoum meet the limitation of a method wherein the gene targeting construct further comprises a first site-specific recombination sequence for a recombinase and a second site-specific recombination sequence for the recombinase, wherein the first and second site-specific recombination sequences flank the DNA encoding the positive selection marker (**claims 2 and 33**), and wherein the recombinase is Cre recombinase (**claims 3 and 34**) and wherein the first and second site-specific recombination sequences are loxP sequences (**claim 4 and 34**) are also discussed.

As discussed above, Capecchi et al teach that the positive selection markers sequences can be neomycin (Neo) and that the negative selection marker can be diphtheria toxin (see column 7, lines 18-20, Table I, in particular), which meets the

limitation of **claims 6, 12, 36 and 39.** Capecchi et al teach that SV-40 can be used as a regulatory sequence, which meets the limitation of **claim 7 and claim 37.**

Capecchi et al teach that that said vector can be used in a method to inactivate genes by homologous integration of the positive selection sequence into the targeted gene and disrupting the expression of the gene in a cell which can be selected by way of the expression of the positive selection marker (column 12, lines 34-52, column 13, lines 6-25 and Example 6, for example) which reads on disruption of a gene of interest in a cell by homologous recombination of the vector and the gene so that the genetically altered cell can be identified by expression of the positive selection markers and meets the limitation of claims **14-15**.

Capecchi et al teach a method comprising producing second transformed target cells by homologous recombination to excise the positive selection marker from the gene (see column 11, lines 1-8, in particular) which meets the limitation of method comprising the step of introducing a recombinase to a first genetically altered cell so that the positive selection marker is removed from the construct to yield a second genetically altered cell (**claim 20**). As described above, Capecchi et al teach embodiment in which the expression of a gene of interest is disrupted by the positive selection marker integrated into the genome (see column 10, lines 49-65), which meets the limitation of a method comprising a step of identifying a first genetically altered cell wherein the genome comprises the construct and the positive selection marker is expressed (**claim 22**). Capecchi et al teach that the second genetically altered cell without the positive selection marker can be further selected and identified (see column

18, lines 9-41, for example) which meets the limitation of a method comprising the step of identifying the second genetically altered cell (**claim 23**).

Capecchi et al exemplify the method in mouse embryonic stem cells, and human bone marrow cells, but disclose that said vectors and methods can be used with any cell type capable of homologous recombination including mammalian and human cells (see column 15, lines 41-55, for example). Capecchi et al further disclose the targeting methods for endothelial cells from human patients (see column 16, lines 41-65, for example), which meets the limitation of somatic cells as mammalian cells and human cells (**claims 16-17 and 24-25**), and the limitation of a cell prepared by the method of claim 18. Sedivy et al contemplate using targeting vectors on highly recombinogenic B cells (see page 89, left column, 4th paragraph, for example), which meet the limitation of a somatic cell as a B cell (**claim 31**).

As discussed above, Barsoum teaches a preferred embodiment in which the carrier DNA is ~300 bp to 1000 bp in length and that sonication may be used to prepare DNA with the desired size distribution (see column 9, lines 44-65, for example). Absent evidence to the contrary, a DNA of 300 bp is at least 75 bp and at least 100 bp and therefore meets the limitation of claims **40-41 and 51-52**.

Claim 49 is rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi et al (of record), in view of Sedivy et al (of record) further in view of Pfarr et al (DNA, 1986, Vol. 5, No. 2, pages 115-122). This is a rejection of a newly added claim.

Applicants claim a somatic cell gene targeting vector comprising wherein the expression cassette comprises a BGH polyadenylation sequence. As discussed above, independent claim 43 from which claim 49 depends, is very similar to cancelled claim 1, with the addition of the limitation of the promoter being a weak promoter or a PGK promoter. Since there is not a specific limiting definition of "weak promoter" in the instant disclosure absent evidence to the contrary, the promoters listed on Table IIB, column 14 of Capecchi meet the limitation of a weak promoter.

The teaching of Capecchi et al and Sedivy et al are discussed in the above rejection. Specifically, Capecchi et al teach that the positive selection marker can in some cases comprise a polyadenylation sequence (see column 13, lines 26-49, in particular) so Capecchi et al contemplate use of polyadenylation sequences. Sedivy et al teach a PNS vector comprising a polyadenylation signal at the 3' end of the positive selection marker and also a polyadenylation signal at the 3' end of the negative selection marker. Sedivy et al also teach a promoterless vector comprising a polyadenylation signal at the 3' end of the positive selection marker (see page 88, Figure 1, in particular). Polyadenylation signals and polyA tails are well known in the art as important structures for transcription and translation.

Capecchi et al do not teach that the polyadenylation sequence is a bovine growth hormone (BGH) polyadenylation sequence. Sedivy et al do not teach that the polyadenylation sequence is a bovine growth hormone polyadenylation sequence.

Pfarr et al teach a comparison of various polyadenylation regions on gene expression in mammalian cells using a downstream galactokinase marker gene. Pfarr

et al teach that a BGH polyA region results in galactokinase expression three times higher than that of SV40 early or human collagen polyA regions (see abstract, in particular).

It would have been obvious to the skilled artisan at the time the invention was made to substitute a BGH polyadenylation for an SV-40 early sequence because Pfarr et al teach that a BGH polyadenylation sequence produces three fold higher gene expression compared to SV-40 early polyadenylation sequence. The motivation to insert polyadenylation sequences is the benefit of being able to get proper transcription and translation of the markers and produce functional selection markers in order to perform the intended gene disrupting method. The motivation to use BGH polyadenylation sequence is the expected benefit of high expression of the selectable marker. There is a reasonable expectation of success in using the claimed polyadenylation sequences in the claimed vector to target somatic cells since this has worked previously in the cited references.

It would also have been obvious to modify the teaching of Capecchi et al to include polyadenylation sequences in the PNS vector because Capecchi et al teach that different regulatory sequences to modify gene expression can be used and combined (column 13, lines 65-67, in particular) and Sedivy et al teaches that polyadenylation sequences flank the positive and negative selection sequences in PNS vectors. There is a reasonable expectation of success in using the claimed polyadenylation sequences in the claimed vector and method to target somatic cells since this has worked previously in the cited references. Given the teachings of the prior art and the level of skill of the

ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, the combination of the teaching of Capecchi et al, Sedivy et al and Pfarr et al renders obvious a vector comprising a BGH polyadenylation sequence.

Claims 11 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi et al (of record), in view of Sedivy et al (of record), in view of Barsoum (U.S. Patent No. 4,956,288) and in view of Pfarr et al (DNA, 1986, Vol. 5, No. 2, pages 115-122). This is a new rejection.

Applicants claim a transfection mixture comprising a somatic cell gene targeting vector comprising a BGH polyadenylation sequence and a double stranded oligonucleotide. Applicants claim a method of gene targeting comprising introducing a gene targeting vector comprising a BGH polyadenylation sequence and a double stranded oligonucleotide.

The teaching of Capecchi et al, Sedivy et al and Barsoum are discussed in the above rejection. Specifically, Capecchi et al teach that the positive selection marker can in some cases comprise a polyadenylation sequence (see column 13, lines 26-49, in particular) so Capecchi et al contemplate use of polyadenylation sequences. Sedivy et al teach a PNS vector comprising a polyadenylation signal at the 3' end of the positive selection marker and also a polyadenylation signal at the 3' end of the negative selection marker. Sedivy et al also teach a promoterless vector comprising a

polyadenylation signal at the 3' end of the positive selection marker (see page 88, Figure 1, in particular). Polyadenylation signals and polyA tails are well known in the art as important structures for transcription and translation. Capecchi et al, Sedivy et al or Barsoum do not teach that the polyadenylation sequence is a BGH polyadenylation sequence.

Pfarr et al teach a comparison of various polyadenylation regions on gene expression in mammalian cells using a downstream galactokinase marker gene. Pfarr et al teach that a BGH polyA region results in galactokinase expression three times higher than that of SV40 early or human collagen polyA regions (see abstract, in particular).

It would have been obvious to the skilled artisan at the time the invention was made to substitute a BGH polyadenylation for an SV-40 early sequence because Pfarr et al teach that a BGH polyadenylation sequence produces three fold higher gene expression compared to SV-40 early polyadenylation sequence. The motivation to insert polyadenylation sequences is the benefit of being able to get proper transcription and translation of the markers and produce functional selection markers in order to perform the intended gene disrupting method. The motivation to use BGH polyadenylation sequence is the expected benefit of high expression of the selectable marker. There is a reasonable expectation of success in using the claimed polyadenylation sequences in the claimed transfection mixture comprising a vector for use in method to target somatic cells since this has worked previously in the cited references.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teaching of Capecchi et al and Sedivy et al and make a transfection mixture comprising a targeting vector and a short carrier DNA oligonucleotide in order to perform a method to disrupt a gene of interest in a somatic cell comprising the step of introducing a short carrier DNA oligonucleotide with the targeting vector in a somatic cell because Barsoum teaches that the efficiency of transfection will be increased by adding a high DNA concentration. The motivation to add a double stranded oligonucleotide is the expected benefit of increasing the total number of integration events and the frequency of high copy number integration events as suggested by Barsoum (see column 10, lines 44-50, in particular). There is a reasonable expectation of success of a method comprising the step of adding a double stranded oligonucleotide to make a transfection mixture in a method to introduce a targeting vector into a cell because it has worked in the cited reference.

Therefore, the combination of the teaching of Capecchi et al, Sedivy et al, Barsoum and Pfarr et al renders obvious the method for disrupting a gene of interest into a somatic cell *in vitro* wherein the second polyadenylation sequence comprises a BGH polyadenylation sequence (**claim 11**). The combination of the teaching of Capecchi et al, Sedivy et al, Barsoum and Pfarr et al also renders obvious the transfection mixture of **claim 38**.

Conclusion

Claims 19 and 42 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Laura McGillem, PhD
Examiner
4/2/2007

DANIEL M. SULLIVAN, PH.D.
PRIMARY EXAMINER